MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2019

April–June 2019—Volume 134, pp. 223–239

https://doi.org/10.5248/134.223

Volvate *Macrolepiota* from Brazil: *M. dunensis* sp. nov., *M. sabulosa* var. *velistellaris* var. nov., and observations on *M. pulchella*

Daline Soares Freitas[1,2] & Nelson Menolli Jr.[1,2*]

[1] *Depto de Ciências e Matemática (DCM) / Biologia,*
Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP),
Câmpus São Paulo, Rua Pedro Vicente 625, 01109-010 São Paulo, SP, Brazil
[2] *Núcleo de Pesquisa em Micologia, Instituto de Botânica,*
Av. Miguel Stefano,3687, Água Funda, CEP: 04301-012, São Paulo, SP, Brazil

[*] Correspondence to: *menollijr@yahoo.com.br*

Abstract—Taxonomic and phylogenetic studies of volvate *Macrolepiota* specimens collected from Atlantic Forest in Northeast and Southeast Brazil are presented based on morphological and molecular (nrITS) data. *Macrolepiota dunensis* and *M. sabulosa* var. *velistellaris* are proposed as new taxa, and additional observations are presented about *M. pulchella*, a species previously known from Brazil. *Macrolepiota dunensis* and *M. pulchella* clustered in the clade corresponding to *M.* sect. *Volvatae*. *Macrolepiota sabulosa* var. *velistellaris* was placed together with non-volvate *M. sabulosa* var. *sabulosa*, close to another volvate taxon (*M. rhodosperma* var. *velicopia*), and together with species classified in *M.* sect. *Macrolepiota*. Our phylogenetic results provide additional evidence that the volva, which is not restricted to members of *M.* sect. *Volvatae*, evolved more than once in the genus.

Key words—*Agaricaceae*, *Lepiotella*, Neotropics, South America, *Volvolepiota*

Introduction

Macrolepiota Singer (*Agaricaceae*) was proposed by Singer (1948) to accommodate specimens characterized by giant basidiospores that are metachromatic in Cresyl blue, a white to cream spore print, and hyphae with clamp connections. Singer (1959) proposed *Volvolepiota* Singer for the illegitimate genus *Lepiotella* Rick (Rick 1938), separated from *Macrolepiota* by the presence of a distinct volva. At present morphological and molecular data (Vellinga 2003; Vellinga & al. 2003) treat *Macrolepiota* and *Volvolepiota* as synonyms, with *Macrolepiota* restricted to specimens that in addition to Singer's diagnostic characters (1948, 1986) possess a trichodermal pileus covering composed of long and often thick-walled elements, a stipe covered with hymeni-trichodermal patches often arrayed in colored bands, and basidiospores with a rounded apex and a germ pore covered by a hyaline cap. The current concept of *Macrolepiota* also includes sequestrate species (Lebel & Syme 2012). Supported by nrITS sequence analyses, Ge & al. (2010) proposed *Macrolepiota* sect. *Volvatae* Z.W. Ge & al. to accommodate volvate species with small amygdaliform-ellipsoid basidiospores and no clamp connections.

Morphological and molecular studies support the existence of only five volvate *Macrolepiota,* distributed mainly in tropical regions: *M. brunnescens* Vellinga from Argentina (Vellinga & Yang 2003; Singer 1959 as *Volvolepiota albida*); *M. eucharis* Vellinga & Halling from Australia (Vellinga 2003); *M. pulchella* from Brazil (Vellinga & Yang 2003, de Meijer 2006; Rick 1938, 1961, as *Lepiotella brunnea*; Singer 1954, 1959, Heinemann & de Meijer 1996, Bononi & al. 1981, as *V. brunnea*); *M. rhodosperma* var. *velicopia* Vizzini & Contu from Italy (Vizzini & al. 2011); and *M. velosa* Vellinga & Zhu L. Yang from China (Vellinga & Yang 2003).

Ge & al. (2010), who included the volvate species *Macrolepiota velosa* and *M. eucharis* in *M.* sect. *Volvatae,* suggested that *M. brunnescens* and *M. pulchella* might also belong to this section. The description of a volvate taxon (*M. rhodosperma* var. *velicopia*) not phylogenetically related to *M.* sect. *Volvatae* representatives (Vizzini & al. 2011) provoked a new discussion about recognizing a natural classification of *M.* sect. *Volvatae* based on velar structures.

The only volvate *Macrolepiota* recorded from Brazil is *M. pulchella*, known from the southern and southeastern states of Rio Grande do Sul (Rick 1938, 1961, as *L. brunnea*; Singer 1954, 1959, as *V. brunnea*), São Paulo (Bononi & al. 1981, as *V. brunnea*), and Paraná (de Meijer 2006; Heinemann & de Meijer 1996, as *V. brunnea*).

Here we report morphological and molecular data for three volvate *Macrolepiota* taxa based on specimens collected in the Atlantic Forest of Rio Grande do Norte (Northeast Brazil) and São Paulo (Southeast Brazil). Two—*M. dunensis* and *M. sabulosa* var. *velistellaris*—are described as new, and we discuss the taxonomy and phylogenetic position of the third, *M. pulchella*.

Materials & methods

Morphological studies

The macroscopic and ecological descriptions are based upon collector's notes from fresh materials. Color terms follow Küppers (1979). For microscopic analyses, sections of lamellae, pileus covering, and stipe were moistened in 70% ethanol and rehydrated in 5% KOH. Reagents used were Congo red to stain hyaline structures, Cresyl blue to test metachromasy, and Melzer's reagent to test basidiospore amyloidy. Microscopic illustrations were made with the aid of a drawing tube attached to an Olympus BX50 compound microscope. For basidiospore measurements, [a/b/c] indicates "a" basidiospores measured from "b" basidioma(ta) taken from "c" collection(s); Q = length/width quotients from all measured basidiospores, Qm = average of all computed Q values; Lm (Wm) = average of lengths (widths) of all measured basidiospores. At least 20 basidiospores from each basidioma were measured in profile view. Basidiospore shape terminology follows Bas (1969), and descriptive terms for other morphological features follow Vellinga (1988) and Vellinga & Noordeloos (2001). The terminology of vegetation types is according to Veloso & al. (1991), and the herbarium acronyms follow Thiers (2018).

Molecular methods and phylogenetic analyses

DNA extraction, PCR, and sequencing methods followed Justo & al. (2011a,b). F The nrITS region was amplified and sequenced using the primer pair ITS-1 and ITS4 (White & al. 1990; Gardes & Bruns 1993). The newly generated sequences were phylogenetically analysed with other DNA sequences of the main representatives of *Macrolepiota* available in GenBank and *Leucoagaricus leucothites* (Vittad.) Wasser and *L. nympharum* (Kalchbr.) Bon as outgroup. GenBank accession numbers and other collection information for the sequences used in molecular analyses are given in Fig. 1. Our alignment matrix, generated by MAFFT 7 (http://mafft.cbrc.jp/alignment/server/; Katoh & Toh 2008) with auto strategy, was visually examined and manually corrected using Geneious 7.0.6 (Kearse & al. 2012).

To identify the best nucleotide evolution model, we used the AICc criterion – Akaike Information Criterion corrected (Hurvich & Tsai 1991) in PartitionFinder 2.1.1 (Lanfear & al. 2017). After separating the ITS region into ITS1+ITS2 and 5.8S, we estimated the evolution model for each partition. Maximum Likelihood (ML) analysis was performed in RAxML v8.2.X (Stamatakis 2014) using the GTR+G model with a rapid bootstrap analysis with 1000 replicates and search for the best-scoring ML tree. The Bayesian inference (BI) analysis was performed with MrBayes v3.2.6 (Ronquist & al. 2012) in the CIPRES Science Gateway 3.1 (Miller & al. 2010) using two partitions with the TVM+G model for ITS1+ITS2 and the JC model for 5.8S, with two independent runs, four simultaneous independent chains, and 10,000,000 generations with a sample frequency every 5000 generation. The following abbreviations are used for statistical data: Bootstrap (BS) and Posterior Probability (PP).

Results & discussion

We would prefer to sample additional collections with a broader morphological variation to support our new taxa and to provide more information about the ontogeny and distribution. Nonetheless, because only five volvate *Macrolepiota* are currently known and because morphological and molecular characters support our material as different taxa, we propose new taxa here to advance *Macrolepiota* research: *M. dunensis* as a new species and *M. sabulosa* var. *velistellaris* as a new variety that distinguished from *M. sabulosa* var. *sabulosa* mainly by the presence of a distinct volva on the stipe base.

Phylogeny

Both ML and BI analyses established the phylogenetic positions of the three Brazilian volvate *Macrolepiota* taxa. The ML tree (Fig. 1) organized the *Macrolepiota* species in three main clades corresponding to the sections indicated by Ge & al. (2010)—*M.* sect. *Macrolepiota*, *M.* sect. *Macrosporae* (Singer) Bon, and *M.* sect. *Volvatae*.

The clade corresponding to *Macrolepiota* sect. *Volvatae* includes sequences from *M. dunensis*, *M. eucharis*, *M. pulchella*, *M. velosa*, and other unidentified collections from Brazil and related to *M. pulchella*. All identified specimens in this clade possess a distinct volva on the stipe base. *Macrolepiota pulchella* clustered with other three sequences of unidentified specimens from Brazil in a well-supported (100% BS, 1 PP) clade (Fazolino Perez & al. 2018), with *M. dunensis* and *M. eucharis* on an external branch. Although we still need to review the morphology of the unidentified collections reported by Fazolino Perez & al (2018) as *Macrolepiota* sp. 3, our molecular results indicate that they are most likely *M. pulchella*. The level of sequence divergence among *M. pulchella* and *Macrolepiota* sp. 3 is up to 1% and is represented by three different base pairs, with an additional different base pair in *Macrolepiota* sp. 3 KY927723 and four ambiguous base pairs in *M. pulchella* MC4411.

The other new volvate *Macrolepiota* (*M. sabulosa* var. *velistellaris*) clusters in the *M.* sect. *Macrolepiota* clade with sequences of the non-volvate typical variety of *M. sabulosa* Fazolino & R.M. Silveira from Brazil (Fazolino Perez & al. 2018), and sequences of *M. cyanolamellata* Fazolino & al. from Brazil (Fazolino Perez & al. 2018) and *M. clelandii* Grgur. from Australia and New Zealand (Vellinga 2003, Vellinga & al. 2003). Although the sequence from *M. sabulosa* var. *velistellaris* is 100% identical to that from the holotype of *M. sabulosa* (and its autonymous variety *M. sabulosa* var. *sabulosa*)—

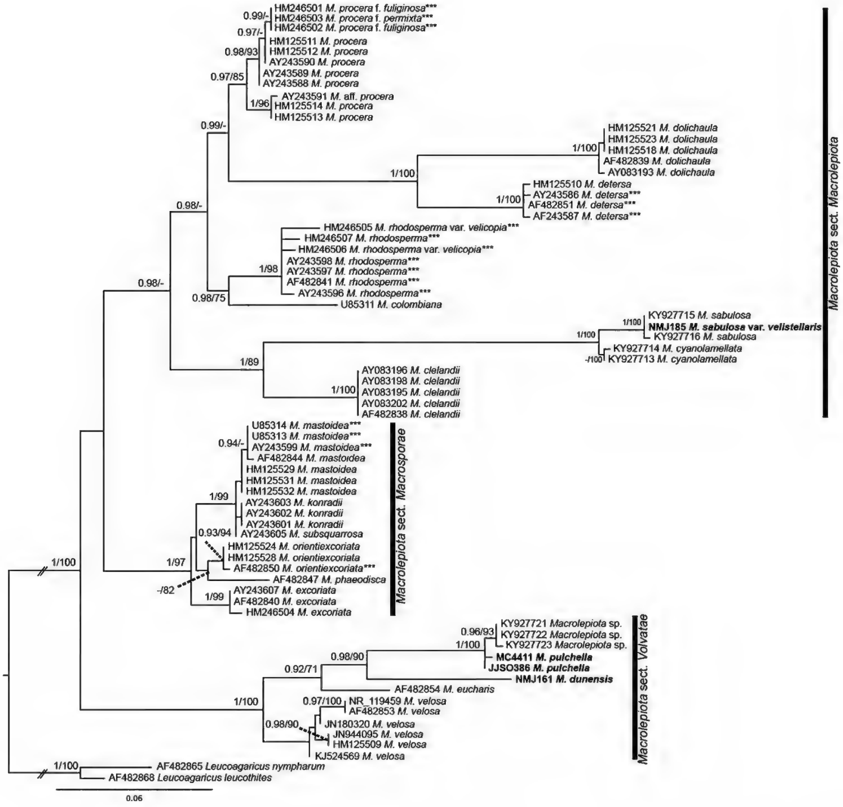

FIG. 1. Bayesian Inference tree of *Macrolepiota* based on nrITS data and rooted with *Leucoagaricus leucothites* and *L. nympharum*. The sequences generated in this work are in bold. Support values (PP >0.90 and BS >70%) are shown above the node branches. *** indicates taxon names modified according to Vizzini & al. (2011).

KY927715, we prefer to propose a new variety based on the morphological differences discussed below.

Vizzini & al. (2011) described another volvate *Macrolepiota* taxon, *M. rhodosperma* var. *velicopia*, which differs from the autonymous variety, *M. rhodosperma* var. *rhodosperma*, primarily by the presence of a volva on the stipe base. *Macrolepiota rhodosperma* var. *velicopia,* which clusters in the *M.* sect. *Macrolepiota* clade, is not phylogenetically related to *M.* sect. *Volvatae*. Vizzini & al. (2011) noted that the presence of a volva on the stipe base is a character that developed independently over the evolution of

Macrolepiota species, suggesting that recognition of *M.* sect. *Volvatae* based solely on the presence of volva is an artificial classification. Additionally, Ge & al. (2010) highlighted the relatively small (usually <15 µm) amygdaliform-ellipsoid basidiospores and absence of clamp connections as additionally diagnostic of *M.* sect. *Volvatae*. The position of *M. sabulosa* var. *velistellaris* outside *M.* sect. *Volvatae* supports the view of Vizzini & al. (2011) regarding the homoplasy of the volva within *Macrolepiota*.

Our sequence analyses also separate *Macrolepiota dunensis* and *M. sabulosa* var. *velistellaris* from all known and sequenced volvate *Macrolepiota* taxa and present for the first time molecular data for *M. pulchella*, the only volvate *Macrolepiota* species previously recorded from Brazil. It is important to note that our phylogeny was generated only from nrITS data and that the internal position and the actual relationships of the sampled taxa may differ when more markers are used.

Taxonomy

Macrolepiota dunensis D.S. Freitas & Menolli, **sp. nov.** FIGS 2A,D, 3

MB823088

Differs from *Macrolepiota pulchella* by its shorter stipe, its lack of triangular patches or squamules on the pileus disc, its smooth and non-encrusted cheilocystidia, and its shorter terminal elements in the pileus covering.

TYPE—Brazil. Rio Grande do Norte: Natal, Parque Estadual Dunas de Natal, Geology trail, 12 July 2010, Menolli Jr. & al. NMJ161 (**Holotype**, SP466944; GenBank MG136892).

ETYMOLOGY—*dunensis* is the Latinized form of dune, for the sand dune area in an Atlantic Forest fragment where the holotype was collected.

PILEUS 50 mm diam., plano-concave, with an obtuse umbo, dark brown ($N_{80}A_{30}M_{50}$) at center, fully covered with appressed fibrils up to two-thirds of the radius, slightly lighter ($N_{80}A_{50}M_{50}$) elsewhere and with the innate fibrils dissociating radially on cream background. LAMELLAE free, ventricose, crowded, cream colored, with lamellulae. STIPE 55 × 3(apex)/7(base) mm, central, brown ($N_{70}A_{60}M_{50}$) and slightly lighter at the apex, covered by inconspicuous zigzag bands mainly on central part, tapering upwards and enlarged at base, fibrous and slightly striate longitudinally. ANNULUS lost but with clear mark on the central stipe. VOLVA limbate, membranous with a cottony base, dirty white to light brown at the margin ($N_{40}A_{30}M_{20}$). CONTEXT, ODOR, TASTE, AND SPORE PRINT COLOR not recorded.

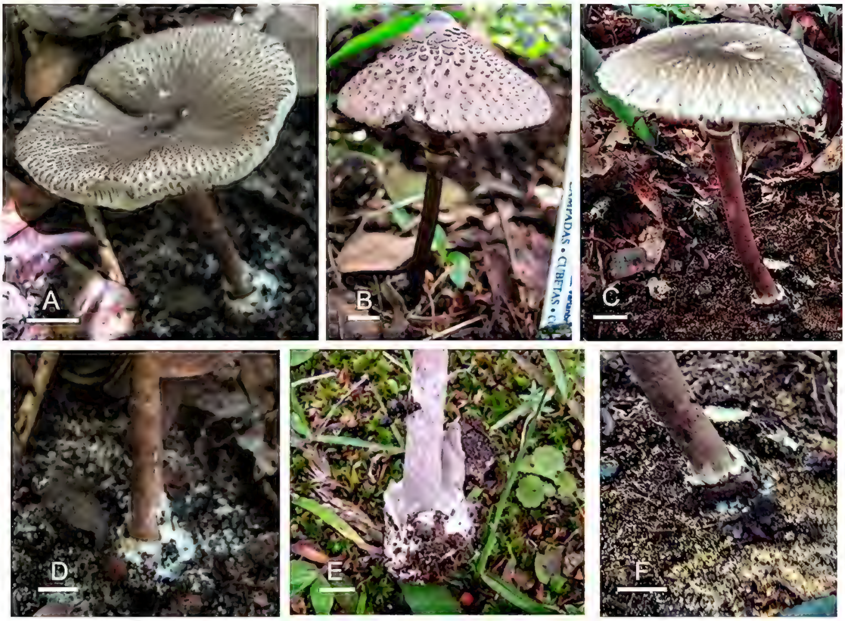

FIG. 2. A, D. ***Macrolepiota dunensis*** (holotype, SP466944). B, E. ***Macrolepiota pulchella*** (B = SP466949; E = SP381606). C, F. ***Macrolepiota sabulosa*** **var.** ***velistellaris*** (holotype, SP466945). Scale bars = 1 cm. Photos: A, D = N. Menolli Jr.; B = J.J.S. Oliveira; C, F = I.G. Baseia; E = M. Capelari.

BASIDIOSPORES [25/1/1] 9.0–12.0 × 6.0–8.0 µm (Q = 1.4–1.8; Qm = 1.6; Lm = 10.7 µm; Wm = 6.8 µm), ellipsoid to elongate, smooth, thick-walled, hyaline, with an apical central truncate germ pore covered by a hyaline cap, some with an inconspicuous apiculus, dextrinoid in Melzer's and metachromatic in Cresyl blue. BASIDIA 26–31 × 11.2–13.0 µm, clavate, hyaline, thin-walled to slightly thickened at the apex, 2-, 3-, and 4-spored in equal abundance. CHEILOCYSTIDIA 32–47 × 7.5–11.2 µm, cylindrical to narrowly clavate, hyaline, thin-walled to slightly thickened towards the apex. HYMENOPHORAL TRAMA irregular composed of thin-walled hyphae, 10.0–16.2 µm diam., hyaline. PILEUS TRAMA hyphae thin-walled, 8.7–16.2 µm diam., with light brown content. PILEUS COVERING an intricate trichoderm, composed of cylindrical elements with light brown content, thin-walled, terminal elements 77–171 × 5.0–7.5 µm. STIPITIPELLIS hyphae thin-walled, 6.2–12.5 µm diam., filled with light brown content. VOLVA hyphae undifferentiated, septate, loosely interwoven, 2.5–10.0 µm diam., thin-walled, colorless to slightly yellowish. NO CLAMP CONNECTIONS seen in all parts examined.

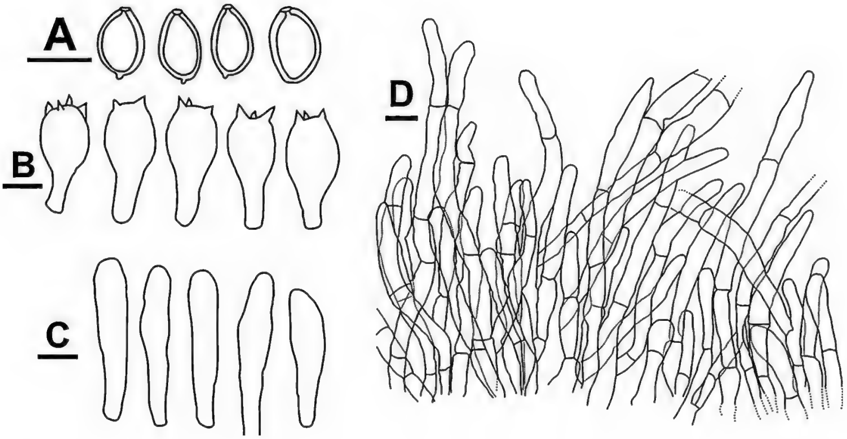

FIG. 3. *Macrolepiota dunensis* (holotype, SP466944). A. Basidiospores; B. Basidia; C. Cheilocystidia; D. Pileipellis elements. Scale bars = 10 μm. Line drawings: D.S. Freitas.

ECOLOGY & DISTRIBUTION—Solitary on sandy soil. In a fragment of shrubby-arboreal stratum on dunes, part of the Atlantic Forest domain in a 'restinga' (tropical coastal vegetation) area.

COMMENTS—*Macrolepiota dunensis* is characterized by a dark brown pileus fully covered with appressed fibrils up to two-thirds from the center and with no velar remnants on the surface, a short stipe, cheilocystidia with slightly thickened walls towards the apex, and short individual terminal elements in the pileus covering. These and other additional morphological characters allied to the phylogenetic position of the sampled taxa (FIG. 1), distinguish *M. dunensis* from the other volvate *Macrolepiota* as discussed below.

Macrolepiota pulchella differs in its pileus with triangular brown-gray squamules at center, longer (≤130 mm) stipe, apical annulus, white membranous volva, slightly shorter (18–40 μm) cheilocystidia with verrucose apices, and pileus covering composed of some pyriform and wider (23–40 μm diam.) hyphae or sometimes walls encrusted with brown pigments (Heinemann & de Meijer 1996).

Macrolepiota eucharis differs in its pileus with black squamules on a brown-gray background, longer stipe (≤140 mm), slightly larger (10.8–15.5 × 7.1–9.1 μm) basidiospores, and pileus surface with shorter and wider (20–80 × 6.0–14 μm) terminal elements (Vellinga 2003).

Macrolepiota brunnescens differs in its broader (<120 mm) pileus partly covered by white woolly-membranous veil remnants, longer (115 mm) white stipe, longer (35–44 µm) basidia that have (sometimes) a brown resinous encrustation or (rarely) brown contents, and an apparent absence of cystidia (Singer 1959).

Macrolepiota velosa differs in its pileus surface with some purplish squamules and white to dirty white universal veil patches, much longer (100–170 mm) purplish brown tinged stipe, longer and narrower (44–68 × 4.5–7.5 µm) cheilocystidia with refractive apices and grayish-granular contents, and pileus covering with wider (25 µm diam.) terminal elements (Vellinga & Yang 2003).

Macrolepiota rhodosperma var. *velicopia* differs in its larger (≤150 mm diam.) pileus with white to dirty white membranous velar patches on the surface; much longer (≤180 mm) stipe; larger ((13–)13.5–15(–16.5) × 9–10.5(–11.2) µm) basidiospores; variously shaped cheilocystidia (narrowly clavate to sub-fusiform, lageniform, utriform, or cylindrical) that are sometimes apically capitulate, basally septate, or (rarely) with apical excrescences; and in the pileus covering much longer (≤250 µm) individual terminal elements with several secondary septa (pseudosepta) and some very thick-walled (1.5–2 µm) hyphae (Vizzini & al. 2011).

Finally, our other newly described taxon, *M. sabulosa* var. *velistellaris*, is distinguished by its slightly rimose pileus surface cracking into very small squamules towards the margin, slightly longer (95 mm) and distinctly squamulose stipe, uniformly thin-walled cheilocystidia, and stipe covering with distinct and ascendant terminal elements (see complete taxonomic description below).

Macrolepiota pulchella de Meijer & Vellinga, Mycotaxon 85:184, 2003. Figs 2b,e, 4

≡ *Lepiotella brunnea* Rick, Lilloa 2: 251. 1938.
≡ *Volvolepiota brunnea* (Rick) Singer, Bol. Soc. Arg. Bot. 8: 12. 1959.
"*Chlorophyllum pulchellum*" Gimenes & Capelari, [unpublished master's thesis]. 2007.

Pileus 32–68 mm diam., conic to convex, light brown ($A_{70}M_{70}C_{60}$; $N_{60}A_{40}M_{40}$) to slightly greyish brown ($N_{80}Y_{50}M_{40}$), dark brown ($A_{99}M_{90}C_{80}$; $N_{90}Y_{50}M_{40}$) on the entire and prominent umbo, margin entire or sometimes with inconspicuous velar remnants, surface completely scaly on fibrillose background and covered with dark brown ($A_{99}M_{70}C_{70}$; $N_{90}Y_{40}M_{40}$) scales (larger at center, smaller toward the edge). Context white, 3.5 mm thick. Lamellae free, remote, crowded, white to light cream ($N_{00}Y_{10}M_{00}$), with lamellulae. Stipe

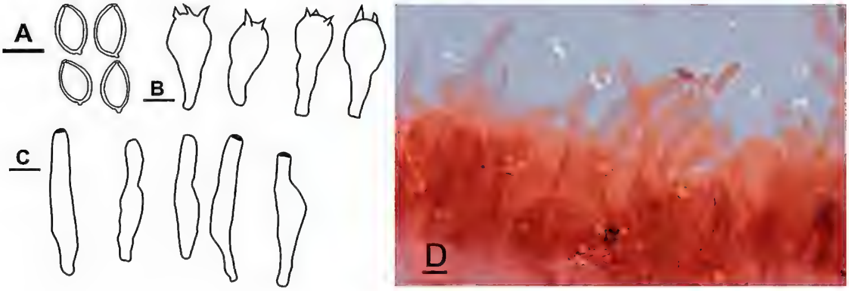

Fig. 4. *Macrolepiota pulchella* (SP466949). A. Basidiospores; B. Basidia; C. Cheilocystidia; D. Pileipellis elements. Scale bars = 10 µm. Line drawings: D.S. Freitas.

75–135 × 3–3.5(apex)/5–10(base) mm, central, dark brown at base and slightly lighter towards the apex, covered by inconspicuous zigzag bands mainly on central part, almost cylindrical or slightly tapering upwards with a subbulbous base, fibrous. Annulus apical, membranous, whitish upperside with brown edge, margin slightly splitting. Volva saccate, membranous with a cottony base, entire white. Odor and taste not recorded. Spore print white.

Basidiospores [187/9/9] (8.7–)9.0–13.7(–15.0) × (6.2–)7.5–10.0(–11.2) µm (Q = 1.1–2.0; Qm = 1.5; Lm = 12.2 µm; Wm = 8.4 µm), subglobose, broadly ellipsoid, ellipsoid, elongate, smooth, thick-walled, hyaline, with a slightly truncate germ pore covered by a hyaline cap, some with inconspicuous apiculus, dextrinoid in Melzer's and metachromatic in Cresyl blue. Basidia 22–40 × 7.5–15.0 µm clavate, hyaline, thin-walled or slightly thickened at apex, 2–3–4-spored. Cheilocystidia 27–55 × 6.2–10.0 µm, cylindrical to slightly clavate, hyaline, thin-walled, some apically encrusted. Hymenophoral trama irregular, hyphae thin-walled, 3.7–16.2 µm diam., hyaline. Pileus trama hyphae thin-walled, 3.7–18.7 µm diam., hyaline to slightly light brown. Pileus covering an intricate trichoderm, composed of thin-walled apically rounded cylindrical elements with light brown contents, terminal elements 72–157 × 5.0–7.5 µm; toward the center intermixed with clavate or pyriform elements. Stipitipellis hyphae thin-walled, 3.7–18.7 µm diam., with brown contents. Volva hyphae undifferentiated, 2.5–8.7 µm diam., thin-walled, septate, loosely interwoven, colorless. Clamp connections not seen in all parts examined.

Ecology & Distribution—Solitary on soil. In a 'seasonal semi-deciduous forest' fragment, part of the Atlantic Forest domain.

Specimens examined—**BRAZIL. São Paulo state: São Paulo:** Itapecerica da Serra, 10 July 1997, A.M. Gugliotta AMG945 (SP466950); 24 July 1997, M. Capelari

> & A.M. Gugliotta MC3955 (SP466951); Parque Estadual das Fontes do Ipiranga, 9 October 2001, U.C. Peixoto PEFI13/2001 (SP381604); 21 September 2005, C. Puccinelli & U.C. Peixoto CP 161 (SP466948); 3 October 2006, C. Puccinelli CP193 (SP381606); 13 August 2006, L.J. Gimenes LJG130/06 (SP381605); 13 November 2007, Menolli Jr. & F. Karstedt NMJ155 (SP466946); 19 October 2011, J.J.S. Oliveira & P.O. Ventura JJSO386 (SP466949; GenBank MG136891); Parque Estadual da Cantareira, 23 October 2008, M. Capelari & L.A.S. Ramos MC4411 (SP466947; GenBank MG136894).

Comments—The morphological features observed in new and old collections confirm the occurrence of *M. pulchella* from São Paulo state, Southeast Brazil, as first noted by Bononi & al. (1981, as *V. brunnea*). However, the collection recorded by Bononi & al. (1981) does not represent *M. pulchella; Macrolepiota* sp. (SP61013) has much larger basidiospores (13.5–16.5 × 9.0–12.0 µm; Q= 1.3–1.6 µm; Qm = 1.4 µm; Lm = 14.9 µm; Wm = 10.6 µm), a longer (approx. 315 mm) stipe, and no evidence of a volva (or any velar remnants) on the stipe base.

In addition to the São Paulo state record, Gimenes (2007) recorded *M. pulchella* (as "*Chlorophyllum pulchellum*") from the same area—Parque Estadual das Fontes do Ipiranga—where we found most of our collections. Gimenes (2007) justified the transfer to *Chlorophyllum* based on an unresolved LSU sequence analysis and the morphology of the pileus covering (described as hymenidermal) and basidiospores (described as lacking the characteristic hyaline cap on the germ pore). However, our revision of the collections studied by Gimenes (2007) plus our examination of new collections and the molecular support confirms the identity of all materials as *Macrolepiota pulchella.*

The most complete morphological study of *M. pulchella* was published by Heinemann & de Meijer (1996) based on a unique basidioma (de Meijer 2935) from Paraná state in South Brazil. They also cited three other collections, drawing attention to minor morphological variations such as the pileus color, context, and squamules; stipe base shape; stability of the annulus; and a volva that can be inconspicuous.

Compared to our *M. pulchella* collections, Heinemann & de Meijer (1996) reported smaller basidiospores ((8.6–)9.41–10.3 × 5.8–6.19(–6.8) µm) and shorter basidia (24–26 µm) and cheilocystidia (18–40 µm), with the cheilocystidia (4–8(–10) µm diam.) sometimes narrowly lanceolate or slightly verrucose at apex. They also described the pileus covering as:

> "... in the center, palisadic, formed of erect hyphae, 5–10 µm diam., with some much larger, e.g. 23–40 µm broad, pyriform elements which are seen at various

> distance from surface; this structure is covered with a layer, 10–35 µm thick, made up of cylindrical, ± radially oriented hyphae, 3–7 µm diam., originating probably from the underlying structure and constituting a general veil; in the periphery, squamules made of cylindrical parallel hyphae, 6–11 µm diam., wall with brown incrusted pigment."

We wish to highlight that among our specimens we observed the characteristic trichodermal pileus covering with long, non-encrusted terminal elements (72–157 × 5.0–7.5 µm). We also noted i) only 2–spored basidia in NMJ155, (23.7–27.5 × 10.0–12.5 µm) similar in size to those described by Heinemann & de Meijer (1996), and basidiospores ((9.5–)10.0–11.5(–12.0) × 7.5–9.0 µm) slightly smaller than the other collections; ii) in JJSO386, the presence of clavate to pyriform elements in the pileus disc covering; and iii) in JJSO386, apically encrusted cheilocystidia possibly corresponding to what Heinemann & de Meijer (1996) described as "slightly verrucose," which, however, does not seem to be a constant character for the species.

Considering that Heinemann & de Meijer (1996) based their description on a single collection, we feel we have accurately described additional morphological characters for *M. pulchella* while providing the first molecular data to contribute to a phylogeny of volvate *Macrolepiota* from Brazil. Additional morphological examinations are needed to confirm the identity of the specimens named by Fazolino Perez & al. (2018) as *Macrolepiota* sp. 3, but our molecular analyses suggest they represent *M. pulchella,* which would be a first record of *M. pulchella* from Rio Grande do Norte state in Northeast Brazil.

Macrolepiota sabulosa var. ***velistellaris*** D.S. Freitas & Menolli, **var. nov.**

MB 823089 FIGS 2C,F, 5

Differs from the non-volvate autonymous variety *Macrolepiota sabulosa* var. *sabulosa* by the presence of a distinct volva on the stipe base.

TYPE—Brazil. Rio Grande do Norte: Baía Formosa, Mata Estrela, Reserva Particular de Patrimônio Natural Mata Estrela, 14 July 2010, Menolli Jr. & al. NMJ185 (**Holotype**, SP466945; GenBank MG136893).

ETYMOLOGY—*veli* (L. for veil), referring to the presence of a volva and velar remnants on the stipe base; *stellaris* (L. for star-shaped), referring to both the star-like arrangement of the pileus squamules and Mata Estrela (Star Forest), the private area where the holotype was collected.

PILEUS 62 mm, plano-convex, with an inconspicuous umbo, dark brown ($N_{80}A_{60}M_{50}$) at center, covering slightly rimose and cracking into very small squamules on one-third distant from the umbo before dissociating radially

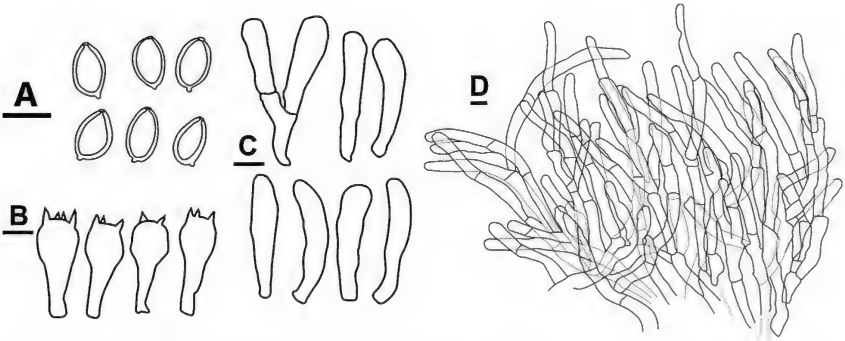

Fig. 5. *Macrolepiota sabulosa* var. *velistellaris* (holotype, SP466945). a. Basidiospores; b. Basidia; c. Cheilocystidia; d. Pileipellis elements. Scale bars = 10 µm. Line drawings: D.S. Freitas.

towards the margin into fibrils on cream background. Lamellae free, crowded, white, with lamellulae. Stipe 95 × 6(apex)/7(base) mm, central, almost cylindrical, covered throughout with small scales forming distinct zigzag bands that concolourous with the pileus disc, lighter at the apex. Annulus apical, membranous, movable, whitish upperside with brown edge. Volva limbate, membranous with a cottony base, dirty white. Context, odor, taste and spore print color not recorded.

Basidiospores [25/1/1] 9.5–12.0 × 6.0–8.0 µm (Q = 1.3–1.8; Qm = 1.5; Lm = 10.7 µm; Wm = 6.9 µm), ellipsoid to elongated, rarely broadly ellipsoid, smooth, thick-walled, hyaline, with distinctive apiculus and an apical central truncate germ pore covered by a hyaline cap, dextrinoid in Melzer's and metachromatic in Cresyl blue. Basidia 29–36 × 11.0–14.0 µm, clavate, hyaline, thin-walled, 2–3–4-spored. Cheilocystidia 25–45 × 7.5–10.0 µm, cylindrical to narrowly clavate, hyaline, thin-walled, sometimes in clusters. Hymenophoral trama irregular, hyphae thin-walled, 5.0–12.5 µm diam., hyaline. Pileus trama hyphae thin-walled, 6.2–11.2 µm diam., hyaline to brownish. Pileus covering an intricate trichoderm, composed of thin-walled cylindrical elements with light brown content, terminal elements measuring 118–193 × 5.0–8.7 µm. Stipitipellis hyphae thin-walled, 5.0–11.2 µm diam., filled with light brown content; terminal elements elongated, conspicuous, ascendant, 6.2–11.2 µm diam. Volva hyphae undifferentiated, septate, moderately branched, loosely interwoven, thin-walled, 3.7–10.0 µm diam., colorless to slightly yellowish. Clamp connections not seen in all parts examined.

Ecology & Distribution—Solitary on soil. In a 'dense ombrophilous forest' fragment within the Atlantic Forest domain.

Comments—*Macrolepiota sabulosa* var. *velistellaris* is proposed as new based on the presence of a distinct volva on the stipe base. Fazolino Perez & al. (2018) described *M. sabulosa* based on two collections from the same state in Northeast Brazil where we collected the new variety. They distinguished *M. sabulosa* from *M. pulchella* due to the presence of a volva on *M. pulchella,* a character neither shown in their illustrations nor mentioned in the protologue of *M. sabulosa*. Additionally, *M. sabulosa* var. *sabulosa* has more robust basidiomata (pileus 130–140 mm, stipe 165–175 × 11–12 mm), shorter terminal elements in the pileus covering (28–63.5 × 5.5–8.5 µm), and clamp connections on hyphal septa in the stipe context and at the bases of basidia, basidioles, and cheilocystidia (Fazolino Perez & al. 2018).

Macrolepiota sabulosa var. *velistellaris* morphologically differs from *M. dunensis* as previously discussed in the notes under *M. dunensis*, as well as from *M. pulchella*, *M. eucharis*, *M. brunnescens*, *M. velosa,* and *M. rhodosperma* var. *velicopia*.

Macrolepiota pulchella has differently arranged pileus squamules, cheilocystidia with a slightly verrucose apex, and a stipe surface composed of brown hyphae (3–9 µm diam.) in a pseudoparenchyma under a palisade structure (no mention of distinct or ascendant terminal elements; Heinemann & de Meijer 1996).

Macrolepiota eucharis is distinguished by its brown-gray pileus background, longer stipe (≤140 mm), slightly larger (10.8–15.5 × 7.1–9.1 µm) basidiospores, mostly 4-spored basidia, slightly fusiform and slightly thick-walled cheilocystidia, and wider (≤14 µm diam.) terminal pileipellis elements (Vellinga 2003).

Macrolepiota brunnescens has a broader (≤120 mm) pileus covered by a woolly whitish membrane, undecorated and initially white stipe, encrusted and pigmented basidia, and (apparently) no cystidia (Singer 1959).

Macrolepiota velosa differs in its purplish tinged pileus squamules, much longer (100–170 mm) stipe, longer and narrower (44–68 × 4.5–7.5 µm) cheilocystidia with a distinct apex, and shorter (≤100 µm) pileipellis terminal elements (Vellinga & Yang 2003).

Macrolepiota rhodosperma var. *velicopia* has a much broader (≤150 mm diam.) pileus covered with white universal veil remnants, much longer (≤180 mm) stipe with a fringed annulus, larger ((13–)13.5–15(–16.5) × 9–10.5(–11.2) µm) basidiospores, differently shaped cheilocystidia (varying among narrowly clavate to sub-fusiform, lageniform, utriform or cylindrical), longer (≤250 µm) pileipellis elements that are normally thick-walled (1.5–2 µm thick) and provided with internal pseudosepta (Vizzini & al. 2011).

Acknowledgments

The authors thank Dr. Alfredo Justo (New Brunswick Museum, Saint John NB, Canada) and Dr. Ana Esperanza Franco-Molano (Universidad de Antioquia, Medellin, Colombia) for critical review of the manuscript; Dr. Shaun R. Pennycook for nomenclature review; Dr. Iuri Goulart Baseia for all the assistance during the expeditions to the state of Rio Grande do Norte; Dr. Tarciso S. Filgueiras (IN MEMORIAM) for help with the Latin names for the new taxa; and Alicia G. Knudson for the English review. They also acknowledge financial support from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo–grant 04/04319-2), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–PNADB grant 23038.000043/2010-43), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico – PIBIC fellowship for D.S. Freitas).

Literature cited

Bas C. 1969. Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. Persoonia 5: 285–579.

Bononi VLR, Trufem SFB, Grandi RAP. 1981. Fungos macroscópicos do Parque Estadual das Fontes do Ipiranga, São Paulo, Brasil, depositados no Herbário do Instituto de Botânica de São Paulo. Rickia 9: 37–53.

de Meijer AAR. 2006. Preliminary list of macromycetes from the Brazilian State of Paraná. Boletim do Museu Botânico Municipal 68: 1–55.

Fazolino Perez E, Suaza Blandón SC, Alves-Silva G, Lechner BE, Silveira RMB. 2018. Taxonomy and phylogeny of *Macrolepiota*: two new species from Brazil. Mycologia 110: 930–940. https://doi.org/10.1080/00275514.2018.1500848

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Ge ZW, Yang ZL, Vellinga EC. 2010. The genus *Macrolepiota* (*Agaricaceae, Basidiomycota*) in China. Fungal Diversity 45: 81–98. https://doi.org/10.1007/s13225-010-0062-0

Gimenes LJ. 2007. A tribo *Leucocoprineae* (*Agaricaceae*) no Parque Estadual das Fontes do Ipiranga, São Paulo, SP, Brasil. [Unpublished Master's Dissertation], Instituto de Botânica de São Paulo. 84 p.

Heinemann P, de Meijer AAR. 1996. The status of *Volvolepiota* Sing. Bulletin du Jardin Botanique National de Belgique 65: 405–412. https://doi.org/10.2307/3668462

Hurvich CM, Tsai C-L. 1991. Bias of the corrected AIC criterion for underfitted regression and time series models. Biometrika 78: 499–509. https://doi.org/10.1093/biomet/78.3.499

Justo A, Vizzini A, Minnis AM, Menolli Jr N, Capelari M, Rodríguez O, Malysheva E, Contu M, Ghignone S, Hibbett DS. 2011a. Phylogeny of the *Pluteaceae* (*Agaricales, Basidiomycota*): taxonomy and character evolution. Fungal Biology 115: 1–20. https://doi.org/10.1016/j.funbio.2010.09.012

Justo A, Minnis AM, Ghignone S, Menolli Jr N, Capelari M, Rodríguez O, Malysheva E, Contu M, Vizzini A. 2011b. Species recognition in *Pluteus* and *Volvopluteus* (*Pluteaceae*, *Agaricales*): morphology, geography and phylogeny. Mycological Progress 10: 453–479. https://doi.org/10.1007/s11557-010-0716-z

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298. https://doi.org/10.1093/bib/bbn013

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Küppers H. 1979. Atlas de los colores: más de 5500 matices con su caracterización y las instrucciones para su mezcla. Editorial Blume, Barcelona.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Lebel T, Syme A. 2012. Sequestrate species of *Agaricus* and *Macrolepiota* from Australia: new species and combinations and their position in a calibrated phylogeny. Mycologia 104: 496–520. https://doi.org/10.3852/11-092

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, Louisiana. 8 p. https://doi.org/10.1109/GCE.2010.5676129

Rick J. 1938. Agarici Riograndenses II. Lilloa 2: 251–316.

Rick J. 1961. Basidiomycetes Eubasidii no Rio Grande do Sul, Brasília. Iheringia 8: 296–450.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Singer R. 1948. New and interesting species of *Basidiomycetes*. II. Papers of the Michigan Academy of Sciences 32: 103–150.

Singer R. 1954. ["1953"] Type studies on *Basidiomycetes* VI. Lilloa 26: 57–159.

Singer R. 1959. Dos generos de hongos nuevos para Argentina. Boletín de la Sociedad Argentina de Botánica 8: 9–13.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th edition. Koenigstein: Koeltz Scientific Books.

Stamatakis A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Strongman DB, Wang J, Xu S. 2010. New trichomycetes from western China. Mycologia 101: 174–184.

Thiers B. 2018 [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Accessed Aug 2018: http://sweetgum.nybg.org/ih/

Vellinga EC. 1988. Glossary. 54–64, in: C Bas & al. (eds). Flora Agaricina Neerlandica, vol. 1. A.A. Balkema Publishers, Amsterdam/Brookfield.

Vellinga EC. 2003. *Chlorophyllum* and *Macrolepiota* (*Agaricaceae*) in Australia. Australian Systematic Botany 16: 361–370. https://doi.org/10.1071/sb02013

Vellinga EC, Noordeloos ME. 2001. Glossary. 6–11, in: ME Noordeloos & al. (eds). Flora Agaricina Neerlandica, vol. 5. A.A. Balkema Publishers, Lisse/Abingdon/Exton/Tokyo.

Vellinga EC, Yang ZL. 2003. *Volvolepiota* and *Macrolepiota—Macrolepiota velosa*, a new species from China. Mycotaxon 85: 183–186.

Vellinga EC, Kok RPJ, Bruns TD. 2003. Phylogeny and taxonomy of *Macrolepiota* (*Agaricaceae*). Mycologia 95: 442–456. https://doi.org/10.1080/15572536.2004.11833089

Veloso HP, Rangel-Filho ALR, Lima JCA. 1991. Classificação da vegetação brasileira adaptada a um sistema universal. Rio de Janeiro: IBGE, Departamento de Recursos Naturais e Estudos Ambientais.

Vizzini A, Contu M, Ghignone S, Vellinga E. 2011. A new volvate *Macrolepiota* (*Agaricomycetes*, *Agaricales*) from Italy, with observations on the *M. procera* complex. Mycotaxon 117: 149–164. http://dx.doi.org/10.5248/117.149

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR Protocols: a guide to methods and applications. San Diego, Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1